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Construction and characterization of a large insert porcine YAC library*

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The recent construction of genetic linkage maps of the porcine genome (Rohrer et al. 1994, 1996; Ellegren et al. 1994; Archibald et al. 1995) allows the assignment of loci affecting heritable traits of economic importance (ETLs; Lander and Botstein 1989) to specific chromosomal segments. Markers can thus be identified that may be useful in marker-assisted selection (MAS) to increase the frequency of favorable allele(s) in resource populations (reviewed in Soller 1994). In addition, mapping of these loci creates the opportunity to identify gene(s) influencing a trait, through positional cloning or positional cnadidate gene approaches (Grootscholten et al. 1991). A positional cloning strategy requires the construction of contigs that physically span large sections of chromosomes. In the human and mouse systems, contig construction has depended on the availability of multiple YAC libraries that provide depth of coverage to minimize the impact of chimeric and deleted clones inherent in these libraries. A single porcine genomic YAC library has been reported (Leeb et al. 1995), but contains only one genome coverage, which limits the ability to make large contigs. We report the construction of a porcine YAC library, with approximately 5.5-fold coverage of the genome and a low rate of chimerism, that provides an additional resource for contig construction and positional cloning.

Preparation of genomic DNA, methylation, restriction, and ligation were performed essentially as described (Larin et al. 1991). Enrichment for large fragments, ligation to vector arms of plasmid pYAC4, and transformations of the *S. cerevisiae* strain AB1380 (Burke et al. 1987) were performed as described (Broom and Hill 1994; Smith et al. 1996). Ura^+ colonies were passaged on Ura^- Trp^-, 20 μg/ml adenine plates, and transferred to YPD media in 96-well plates (Falcon) in triplicate for storage (−80°C). The library was gridded onto filters with a Biomek 1000 work station (Beckman) as described (Bentley et al. 1992; Broom and Hill 1994). Clones were pooled with 1440 clones in each primary pool (total of 23 pools), and secondary pools (rows and columns) of 120 or 180 clones. Currently, the porcine YAC library consists of 345 plates containing approximately 33,120 individual clones.

Overall library coverage was assessed by Southern analysis of insert size. Agarose plugs containing total yeast DNA from individual YAC clones were prepared and subjected to pulsed field gel electrophoresis (PFGE), transferred to nylon membranes, and probed with radiolabeled sheared total porcine DNA (Sambrook et al. 1989). A histogram of the results of this analysis is shown in Fig. 1A. The mean insert size of 152 clones analyzed was 589 kb, with a median size of 480 kb. Thus, the predicted level of coverage is theoretically 5.3 (median) to 6.5 (mean) genome equivalents, assuming a 3 billion bp genome.

The number of genome equivalents in the library was more rigorously defined by subjecting the 23 primary DNA pools to PCR analysis with 52 primer pairs representing markers from each of the 18 autosomal linkage groups, and the X Chromosome (Chr) (Rohrer et al. 1994, 1996; Alexander et al. 1996a, 1996b). PCR reactions contained 300 ng of primary pool total yeast DNA, and conditions were as described (Rohrer et al. 1994; Alexander et al. 1996a, 1996b). The number of primary pools for which a specific size fragment was produced was assumed to be equivalent to the number of YAC clones containing the target sequence. A mean value of 5.5 positive pools per primer pair was observed, with a range of 2-11 (Fig. 1B). The mean represents a minimum estimate of the number of genome equivalents, since analysis of secondary pools for five of the primers revealed that some primary pools contain more than one positive clone. Therefore, actual coverage is greater than 5.5 genome equivalents, a figure within the theoretical range predicted from the average insert size of the clones (5.3–6.5).

The level of chimerism was assessed by FISH analysis. YAC DNA was separated by PFGE, excised, biotinylated, and hybridized to porcine metaphase spreads as described (Lichter et al. 1990). In total, 28 clones were analyzed in the size range from 225 to 825 kb. Twenty were observed to hybridize to single chromosomal locations. Two of the chimeric clones were in the size range 225 to 300 kb, and the remaining six chimeric clones were larger than 565 kb. Although the level of chimerism is based on a small number of clones, the level of chimerism (28.5%) is consistent with the level observed in sheep (Broom and Hill 1994) and bovine YAC (Smith et al. 1996) libraries constructed with identical procedures.

We have produced a porcine YAC library of sufficient redundancy and low level of chimerism to be useful for a variety of purposes, including positional cloning of an ETL. We have directly shown that the library has overall 5.5-fold coverage of the genome and includes markers from all 18 autosomes and the X Chr. This makes it the most extensively characterized porcine library available.

The library represents a public resource for fine physical mapping in the porcine genome. The PCR screening process can isolate multiple YAC clones carrying a specific marker, or set of markers, in one working week, making the integration of the swine physical and genetic maps highly efficient. The availability of YAC probes for selected positions on the various porcine chromosomes opens the possibility of ordering loci by multicolor fluorescence on metaphase or interphase chromosomes, producing chromosomal "bar codes" as demonstrated in the human system (Lengauer et al.

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*Proprietary or brand names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may be suitable.

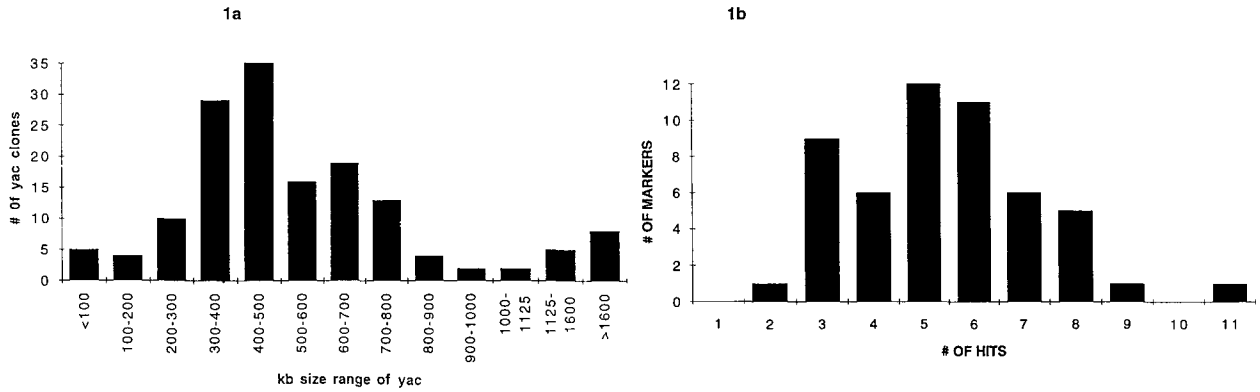

Fig. 1. Assessment of coverage of the porcine YAC library: **(a)** Histogram showing the insert sizes of 152 randomly selected YAC clones. **(b)** PCR analysis of 52 porcine microsatellite primer pairs. The 23 primary pools were screened for positive clones with microsatellite primers from all 18 autosomes and the X Chromosome.

1993). We believe the public availability of this library, in conjunction with the high-resolution genetic linkage maps (Rohrer et al. 1996), represents an important resource for the porcine mapping community. Primary and secondary PCR pools will be made available for screening upon request to the authors.

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